

**Evaluation of Recombinant Herpes Zoster Vaccine for Primary Immunization of
Varicella-seronegative Transplant Recipients**

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DISCLAIMER

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

The trial was registered at clinicaltrials.gov (NCT03685682).

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Abbreviations :

ACIP : Advisory Committee on Immunization Practices ; AST : American Society of Transplantation ; CDC : Centers for Disease Control and Prevention ; DEA: diethylamine ; DMSO : dimethyl sulfoxide ; ELISA : enzyme-linked immunosorbent assay ; gE : glycoprotein E ; gp : glycoprotein ; HZ : herpes zoster ; IDSA : Infectious Diseases Society of America ; IQR : interquartile range ; NIH : National Institutes of Health ; OD: optical density ; PBMC : peripheral blood mononuclear cells ; PBS: phosphate buffered saline; RZV : recombinant subunit herpes zoster vaccine ; SOT : solid organ transplantation ; SPICE : Simplified presentation of incredibly complex evaluations ; VZV : varicella-zoster virus

ABSTRACT

Background: Immunization of VZV-seronegative solid organ transplant (SOT) patients using the live-attenuated varicella vaccine is generally contraindicated, leaving no widely applicable immunization option. The recombinant subunit herpes zoster vaccine (RZV) is indicated for VZV seropositive persons to prevent shingles but could potentially also protect VZV-seronegative persons against varicella. We performed a safety and immunogenicity evaluation of RZV in VZV-seronegative SOT recipients as an option for protection.

Methods: VZV-seronegative adult SOT patients with no history of varicella/shingles vaccine or disease were given 2 doses of RZV vaccine 2-6 months apart. Blood was drawn prevaccination (V1), prior to the second dose (V2) and 4 weeks after second dose (V3). Humoral (anti-gE) and cell-mediated immunity was evaluated, with polyfunctional cells defined as cells producing ≥ 2 cytokines.

Results: Among 31 eligible VZV-seronegative SOT patients screened, 23 were enrolled. Median age was 38 years and median time since transplant procedure was 3.8 years. The most frequent transplant types were liver (35%) and lung (30%). Median anti-gE levels significantly increased from V1 to V3 ($p=0.001$) and V2 to V3 ($p<0.001$), even though only 55% had a positive seroresponse. Median polyfunctional CD4 T-cells counts increased from V1 to V2 ($54/10^6$ vs $104/10^6$ cells; $p=0.041$), and from V2 to V3 ($380/10^6$; $p=0.002$). Most adverse events were mild with no rejection episodes.

Conclusion: RZV was safe and elicited significant humoral and cellular responses in VZV-seronegative SOT patients, and has the potential to be considered as a preventive strategy against primary varicella.

INTRODUCTION

Solid organ transplant (SOT) recipients are at increased risk for severe or complicated primary varicella infection (chickenpox) because of the lifelong immunosuppression essential to preventing organ rejection.^{1,2} Compared to transplanted children, adult seronegative SOT recipients seem to have an increased rate of complications and mortality when exposed to varicella-zoster virus (VZV).³ Similarly, SOT patients are at increased risk for complicated shingles (herpes zoster [HZ] and postherpetic neuralgia in case of VZV reactivation.^{4,5} The current vaccine for preventing primary VZV infection is a live-attenuated virus vaccine. Because of the risk of vaccine-induced chickenpox, the Infectious Diseases Society of America (IDSA) and the American Society of Transplantation (AST) both contraindicate the live varicella vaccine after SOT, but emphasize the importance of immunizing seronegative candidates with the live vaccine at least 4 weeks prior to SOT.^{6,7} Unfortunately, optimizing VZV immunization prior to SOT is not always feasible because of the urgency of some transplants or if the candidate is already immunosuppressed for underlying conditions. Therefore, many transplanted patients are left unvaccinated against primary varicella infection.

Shingrix (GSK Vaccines) is recommended by the US Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) for the prevention of shingles in persons ≥ 50 years of age. Shingrix is a nonlive, recombinant subunit zoster vaccine (RZV) that contains 50 μ g lyophilized varicella zoster virus glycoprotein E (gE) antigen with the accompanying AS01B adjuvant. The vaccine has been shown to be effective in preventing zoster in adults ≥ 50 years old in a large phase III randomized controlled trial, with an overall vaccine efficacy of 97.2%.⁸ Because it is a subunit vaccine containing only a single viral protein (gE), it has also been studied in VZV seropositive immunocompromised populations. In a placebo-controlled RCT, the vaccine has been shown to induce cellular and humoral immunity in VZV IgG-positive kidney transplant patients.⁹ It was also shown

immunogenic in persons living with HIV and is efficacious in preventing shingles in seropositive autologous stem cell transplants.^{10,11}

RZV has been shown to induce humoral and cellular immunity,⁹⁻¹³ both of which should contribute to also protect against primary VZV infection. Although the primary indication for this vaccine is to boost immunity in VZV IgG-positive persons, we hypothesized that the vaccine should be able to induce immunity in VZV IgG-negative patients. To date, there are limited data about RZV among VZV IgG-negative patients. The aim of our study was to evaluate whether RZV elicited cellular and humoral immunity in a cohort of VZV-seronegative SOT patients and to determine the feasibility of this approach. RZV could be an option to optimize protection against VZV among SOT patients who had not received the live VZV vaccine before SOT. This could also be applied to other immunosuppressed cohorts who cannot receive the live vaccine.

MATERIALS AND METHODS

2.1 Patient population and study design

This prospective interventional study was conducted starting June 2018, at the University Health Network Transplant Centre, a tertiary care organ transplant program in Toronto, Canada. Inclusion criteria were 1) age ≥ 18 years old, 2) history of organ transplantation (kidney, liver, heart, lung, pancreas, intestine or combined) 3) functioning allograft, 4) at least 90 days following transplant and 5) negative VZV IgG at time of transplant and confirmed prior to vaccination. Exclusion criteria were 1) prior history of shingles or chickenpox (occurring either before or after SOT), 2) positive or equivocal VZV IgG at any time before or after SOT, 3) prior VZV or HZ immunization, 5) ongoing CMV viremia > 200 IU/mL, 6) other immunodeficiency such as HIV positive, 8) treatment for rejection in the past 30 days, 9) immunoglobulin (eg IVIg) in the past 30 days or anticipated to receive immunoglobulin. The study was approved by the institutional research ethics board as well as Health Canada

(#215830) due to off-label use of RZV. The study was registered at clinicaltrials.gov (NCT03685682).

After obtaining consent, participants received 2 intramuscular 0.5mL doses of RZV 2 to 6 months apart. Serum and PBMCs were collected prior to first dose of vaccine (V1), prior to second dose of vaccine (V2), and 4 weeks after the second dose (V3) (Figure 1). A negative prevaccination serostatus was confirmed by the VIDAS ELFA (enzyme linked fluorescent assay) VZV IgG assay (VIDAS, Biomerieux, France). Patients were followed up to 3 months after the second vaccine dose.

2.2 Outcomes

The primary outcomes were gE-specific cellular and humoral immunogenicity. The secondary outcome was the safety of the vaccine in the VZV-IgG negative SOT population. A positive seroresponse was defined as an antibody level (based on optical density; see below) greater than 2 standard deviations above the mean prevaccination value.

2.3 Humoral immunity

Sera from all 3 time-points were used for evaluation of humoral immunity using 2 enzyme-linked immunosorbent assays (ELISA), one specifically targeting VZV glycoprotein E (anti-gE) and the other a gpELISA (using lentil lectin-purified VZV glycoproteins (gp) including gE, gB, gH).¹⁴ Both gpELISA and gE ELISA assays were performed at the VZV reference laboratory at the Centers for Disease Control and Prevention (CDC, Atlanta, USA). Samples were tested in duplicate. Results for gpELISA were expressed as adjusted optical density (OD) values (mean test OD – mean normal tissue control OD). Cut-off point and performance specifications for gpELISA were determined on prevaricella and postvaricella serum samples from 12-18-month old children (preimmunization = true negative; postimmunization [>3 weeks] = true positive). Since the gE ELISA utilizes a highly purified glycoprotein E as the target antigen, no normal tissue control was required. gE ELISA was compared with gpELISA and found to be in substantially complete agreement. Antibody avidity (binding

strength of antibody) was determined as follows: 2 identical ELISA run plates were prepared, 1 of which was washed using phosphate buffered saline (PBS) and the other with PBS containing diethylamine (DEA). The avidity index (%) was calculated using the OD of wells washed with PBS-DEA divided by the OD of wells washed with PBS alone, multiplied by 100 (Range of results: high avidity ≥ 60 , moderate avidity 30 to 59; low avidity ≤ 29). For statistical purposes, a positive seroresponse was defined as an OD value greater than 2 standard deviations above the mean of prevaccination values.

2.4 Cellular immunity

Evaluation of gE-specific cellular immunity was performed using intracellular cytokine staining and flow cytometry. Peripheral blood mononuclear cells (PBMCs) were extracted from whole blood using Ficoll gradient centrifugation (GE Healthcare Life Science, Issaquah, WA, USA) and cryopreserved in fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) with 10% dimethyl sulfoxide (DMSO) (Fisher BioReagents, Thermo Fisher Scientific). To determine vaccine-associated responses, 1×10^6 PBMCs were stimulated immediately after thawing with $1.25 \mu\text{g/mL}$ of a VZV peptide pool (JPT Peptide Technologies, Berlin, Germany) or with media alone for 2 hours. As VZV peptides were reconstituted with DMSO, DMSO was added at the same concentration to the unstimulated specimens as a negative control. The BD FastImmune anti-human CD28/CD49d costimulatory reagent (BD Biosciences, Mississauga, Canada) was added at the same time as the antigen. After 2 hours of incubation at 37°C , Brefeldin A Solution (protein transport inhibitor) (BioLegend, San Diego, CA) was added at a concentration of $1 \mu\text{g/mL}$ for another 18 hours. Following stimulation, cells were centrifuged and stained with the Zombie Aqua viability dye (BioLegend). Following Fc receptor blocking using Human BD Fc Block (BD Biosciences), cells were incubated with a cell-surface cocktail consisting of mouse anti-human CD45 (clone HI30)-PerCP/Cy5.5 (Biolegend), mouse anti-human CD3 (clone OKT3)-BV786 (BD Horizon, BD Biosciences, mouse anti-human CD4 (clone RPA-T4)-Pacific Blue

(BD Pharmingen, BD Biosciences) and mouse anti-human CD8 (clone SK1)-APC-Cy7 (BD Pharmingen, BD Biosciences). Following incubation with fixation buffer (BioLegend) cells were treated with an intracellular cytokine antibody cocktail prepared in permeabilization wash buffer (BioLegend). The intracellular cocktail consisted of mouse anti-human interferon (IFN)- γ (clone B27)-FITC (BD Pharmingen, BD Biosciences), mouse anti-human tumor-necrosis factor (TNF)- α (clone MAb11)-PE-Cy7 (BD Pharmingen, BD Biosciences), rat anti-human interleukin (IL)-2 (clone MQ1-17H12)-APC (BD Pharmingen, BD Biosciences) and mouse anti-human CD154 (CD40L) (clone 24-31)-PE (BD Pharmingen, BD Biosciences). Flow cytometry was performed on a BD LSR II (BD Biosciences) at The SickKids-UHN Flow and Mass Cytometry Facility (Toronto, ON, Canada) with a target event count of 100 000 live, CD45⁺ cells. Data were analyzed using FlowJo software v10 (FlowJo LLC, Ashland, OR). To account for background cytokine production, the frequency of cytokine-producing T-cells obtained in the unstimulated specimen was subtracted from the frequency obtained in the stimulated specimen. Cells producing at least 2 cytokines among TNF- α , IFN- γ , IL-2 and CD40L were considered polyfunctional and were determined as previously described.¹⁵ A representative gating strategy for the identification of cytokine-producing CD4⁺ and CD8⁺ T-cells is presented in Figure S1 <http://links.lww.com/TP/C105>.

2.5 Safety

Patients were provided questionnaires to complete regarding local (pain, redness, swelling) and systemic (fever, fatigue, headache, myalgia, gastrointestinal symptoms) adverse events during the first 7 days after each vaccine dose. Adverse events were categorized as mild (no interference with daily activities), moderate (some interference in daily activities) and severe (significant interference with daily activities). Other adverse events were recorded using chart review. Biopsy-proven or clinically-treated episodes of rejection were recorded via chart review until 90 days after the last vaccine dose.

2.6 Statistical Analysis

Analyses were performed in patients for which both baseline and postvaccine specimens were available, per-protocol. For group comparisons, Chi-squared or Fisher exact test were used for dichotomous variables, whereas Mann-Whitney U test was used for continuous variables. For paired analysis, the Wilcoxon signed rank test was used and Kruskal-Wallis test was used to compare across mycophenolate dosing groups. Correlations were determined using Spearman coefficient. A line of best fit was generated using simple linear regression to show the relationship between humoral and cellular immunity. P-values <0.05 were considered statistically significant. Statistics were performed using SPSS version 23.0 (IBM Corp., Armonk, NY). Figures were made using GraphPad version 7.0 (La Jolla, CA) or National Institutes of Health (NIH)'s Simplified presentation of incredibly complex evaluations (SPICE). Daily doses of MMF were converted into mycophenolate equivalent, as previously described.¹⁶

RESULTS

3.1 Demographics

Among approximately 5000 SOT patients followed in our transplant program, 57 were VZV-IgG negative. Of those, 31 met inclusion criteria and were approached (Figure 2). Twenty-three patients consented to participate, 7 did not consent and 1 patient was not enrolled because of a concurrent acute kidney insufficiency under investigation. Every patient enrolled was VZV seronegative prior to transplant and had VZV IgG retested at V1 and were confirmed IgG negative. All 23 patients completed the study (Figure 2).

Median age at enrollment was 38 years and median time between SOT and enrollment was 3.8 years (Table 1). The median interval between the 2 vaccine doses was 2.7 months (interquartile range [IQR] 2.3-3.5). Liver transplant recipients were the most frequently enrolled patients, followed by lung and then kidney transplant. Most (78.3%) patients were on

triple immunosuppression, whereas 8.7% and 13.0% were on double and single immunosuppression, respectively.

3.2 Humoral immunity

Anti-gE. In the cohort of 23 patients, median anti-gE levels significantly increased from V1 to V3 ($p=0.001$), and V2 to V3 ($p<0.001$), but not from V1 to V2 ($p=0.237$) (Figure 3). Three patients had baseline serology thresholds above the cut-off as measured by gpELISA, despite having negative VZV IgG by ELISA. Excluding these 3 patients, median anti-gE levels showed a similar increase from V1 to V3 ($p<0.001$), V2 to V3 ($p<0.001$) but not V1 to V2 ($p=0.449$) (Figure S2 <http://links.lww.com/TP/C105>). In these 20 patients, only 11 (55%) had a positive seroresponse to the vaccine. Median anti-gE avidity ($n=23$) increased from V1 (0% [IQR 0%-0%]) to V2 (0% [IQR 0%-10%]; $p=0.025$), V1 to V3 (12% [IQR 0%-60%]; $p=0.002$) and V2 to V3 ($p=0.006$).

gpELISA. Median VZV antibody levels by gpELISA significantly increased from V1 to V3 ($p=0.009$), and V2 to V3 ($p=0.003$), but not from V1 to V2 ($p=0.162$) (Figure 3). There was a strong correlation between anti-gE and gpELISA antibodies ($r=0.947$; $p<0.001$) after 2 vaccine doses (Figure S3 <http://links.lww.com/TP/C105>).

Factors associated with humoral immunity. There was no difference in median anti-gE levels at V3 between lung and non-lung transplant recipients ($p=0.169$). The use of prednisone and mycophenolate (analyzed as yes/no variables) was associated with significantly lower anti-gE responses ($p=0.046$ and 0.004 respectively), whereas no association was noted with tacrolimus or cyclosporine (data not shown). However, there was no correlation between anti-gE levels and the dose of either prednisone ($r=0.048$; $p=0.854$) and mycophenolate ($r=0.038$; $p=0.884$).

3.3 Cell-mediated immunity

Median polyfunctional CD4⁺ T-cells counts significantly increased from V1 (54/10⁶ cells [IQR 27-97/10⁶]) to V2 (104/10⁶ cells [IQR 47-271/10⁶]; p=0.041), from V2 to V3 (380/10⁶ cells [IQR 99-1034/10⁶]; p=0.002) and from V1 to V3 (p<0.001) (Figure 4). There was no significant increase in median polyfunctional CD8⁺ T-cells between V1 (43/10⁶ cells [IQR 17-99/10⁶], V2 (39/10⁶ cells [IQR 24-85/10⁶]) and V3 (74/10⁶ cells [IQR 30-133/10⁶]) (Figure 4). Among polyfunctional CD4⁺ T-cells, IL-2 and CD40L contributed the most to polyfunctionality (Figure S4 <http://links.lww.com/TP/C105>).

Factors associated with cell-mediated immunity. Lung transplant recipients had lower median polyfunctional CD4⁺ T-cells counts at V3 (99/10⁶ [IQR 64-256]) than other organ transplant recipients (540/10⁶ [IQR 207-1350]; p=0.015). The use of individual immunosuppressants including prednisone (as a yes/no variable) were not associated with VZV-specific cellular immunity (data not shown). However, increasing doses of mycophenolate was associated with lower polyfunctional CD4⁺ T-cell responses at V3 (r=-0.593; p=0.009). The effect of individual doses of mycophenolate is shown in Figure S5 <http://links.lww.com/TP/C105> .

Correlation between humoral and cell-mediated immunity. Anti-gE titers and polyfunctional CD4 T-cells at V3 were moderately correlated (r=0.515; p=0.014) after the second vaccine dose (Figure S6 <http://links.lww.com/TP/C105>).

3.4 Safety

The vaccine was overall well-tolerated. Questionnaires were returned after 38/46 vaccine doses (83%). Among solicited adverse events, pain at injection site and myalgia were the most frequently reported (after 76% and 42% of doses, respectively) (Table 2). Ninety-one percent of the adverse events were mild or moderate. All events were self-limited and no medical attention was needed. There were 17 unsolicited adverse events that occurred in 10 patients. None were evaluated as being related to vaccination. These included 9 admissions

for the following reasons: fever or infectious syndromes (n=3), cardiac (n=2), liver failure (n=2, including 1 death), new onset inflammatory bowel disease (n=1) and deep venous thrombosis (n=1). The 2 admissions for liver failure happened in the same liver transplant patient known prior to enrollment for a failing graft secondary to recurrence of primary sclerosing cholangitis. Other adverse events managed in the ambulatory setting were infectious (n=4), renal (n=1), ophthalmologic (n=1), chest pain (n=1) and enlarged lymph node (n=1). No rejection occurred in the 3 months following vaccination.

DISCUSSION

We performed a proof-of-concept study to determine the immunogenicity and safety of 2 doses of recombinant zoster vaccine in a cohort of VZV seronegative transplant recipients. We found that a significant proportion of patients develop gE-specific humoral and cell-mediated immunity suggesting that this is a viable option for primary vaccination of VZV seronegative patients who have a contraindication to live VZV vaccine. The vaccine was also well-tolerated, with frequent though mostly mild or moderate adverse events. To our knowledge, there are no prior published data on the immunogenicity and safety of RZV in VZV-seronegative cohorts.

Vaccine options for immunocompromised patients that are susceptible to primary varicella are limited. Patients can receive live varicella vaccine in the pretransplant period but this has generally been avoided since patients need to be put on hold for transplant for 4 weeks after each vaccine dose. Recently there has been an effort to select patients posttransplant that are on low levels of immunosuppression and could receive live vaccines.¹⁷ However, these guidelines may not apply to a significant proportion of patients and carries inherent risks related to live virus vaccination in immunocompromised patients. Based on immunosuppression, almost all of our adult study cohort (22/23) would not meet the criteria for safe live-attenuated vaccine administration recently suggested for transplanted children.¹⁷ RZV is a recombinant subunit inactivated vaccine and has previously been studied in

immune-competent as well as immunocompromised adults who are VZV-seropositive. In this setting, it has good immunogenicity and efficacy to prevent shingles.⁸⁻¹³ However, it is unknown whether RZV could potentially prevent primary varicella infection. RZV contains recombinant gE protein and is mixed with an adjuvant prior to administration. Glycoprotein E plays a critical role in many aspects of the viral life cycle including viral entry into the cell and virion assembly.^{18,19} Therefore, in theory, it can be postulated that humoral immunity to gE may prevent primary infection or may mitigate its severity. Although the prevalence of VZV-naïve individuals is generally low in the adult transplant population (<5% in the literature and 1-2% at our transplant center),²⁰ it is significantly greater in pediatric transplantation where immunizations may be missed due to chronic illness.²¹ We evaluated humoral immune response to the vaccine using various methods: gE ELISA including antibody avidity and gpELISA. Overall, there was a significant increase in antibodies although the rise was only significant after the second vaccine dose suggesting that at least 2 doses are required for a robust humoral response. Given that just over half the patients met the criteria for humoral response, it is possible that more than 2 doses would be required in a VZV-naïve immunosuppressed population. Humoral immunity was expressed as mean (adjusted) in optical density rather than an antibody concentration since international standard sera for these assays were not available. Moreover, correlates of protection for anti-gE are not defined. Nevertheless, we found that based on our conservative definition of vaccine response, more than half the transplant patients had a humoral response. Anti-gE also highly correlated with results from gpELISA. The gpELISA is readily available through reference laboratories and has greater sensitivity for evaluation of humoral immunity postvaricella vaccination than a standard VZV IgG.²² Although not specific to gE, this could also potentially be useful for determine response to the subunit zoster vaccine since anti-gE assays are not widely available. Antibody avidity was low likely due to immunosuppression.

Notably, we found that 3 patients who were VZV IgG seronegative by local testing, had a positive baseline gpELISA result. Even if these patients are excluded, antibody response to gE was significant in the remaining cohort. T-cell immunity also plays a critical role in control of herpesviruses. Therefore, we used a VZV gE peptide pool to stimulate PBMCs. Interestingly, although CD4⁺ T-cells significantly increased after vaccination, a CD8⁺ T-cell response was not seen and this finding is in keeping with previous T-cell studies of RZV vaccination.^{10,13} It is possible that this is due to the antigenic stimulus used in the in vitro assay and may differ if other antigens such as VZV lysate were used. Interestingly, CD4⁺ T-cells responses have been shown to be more important than CD8⁺ responses in the control of varicella.^{23,24} Baseline T-cell reactivity against gE protein was found in many patients; since patients were VZV-naïve, this may be due to crossreactivity of VZV gE epitopes with that of HSV-related gE. HSV seropositivity is common in the general population and HSV also encodes a gE protein similar to VZV-gE.²⁵ An alternate explanation is T-cell receptor promiscuity with response to heterologous antigens.²⁶ Nevertheless, there was significant rise in CD4⁺ T-cell responses postimmunization. The humoral response had a moderate and significant positive correlation with the T-cell response.

Immunosuppression had an important impact on vaccine responses and the current study shows that prednisone and mycophenolate both inhibited anti-gE responses whereas only mycophenolate inhibited VZV-specific T-cell responses. Prednisone inhibits antigen presentation and mycophenolate is a cell cycle inhibitor that also has a dose-dependent effect on humoral and cellular responses to influenza vaccine in transplant recipients.^{27,28}

RZV contains the AS01B adjuvant and general concerns have been raised regarding enhanced alloimmunity and autoimmunity with adjuvants. We did not find any significant rejection episodes that occurred after vaccination; however, most patients were several years posttransplant, when the risk of rejection is lower. Anecdotal evidence and small case series from the adjuvanted influenza vaccine literature have suggested that adjuvanted vaccines

could induce the production of donor specific anti-HLA antibodies.²⁹ However, in larger samples, these vaccines proved to be safe with regards of the risk of rejection in solid organ transplant recipients.^{30,31} Moreover, in a randomized trial of kidney transplant recipients that were given RZV vaccine, there was no increase in risk of rejection compared to placebo.⁹ We did not test for HLA antibodies due to lack of a control group but future studies could compare changes in HLA alloantibody in a controlled setting.

Our study has some limitations. The sample size was relatively small and reflects the small numbers of VZV IgG negative transplant recipients at a given transplant center; however, we were able to demonstrate immunogenicity and safety of the vaccine, albeit in a predominantly white population. The concentration of anti-gE was not determined making it difficult to compare anti-gE levels to previous immunogenicity studies with RZV. However, correlates of protection with anti-gE are not known and we were able to demonstrate a significant rise in anti-gE levels after the second dose. Our study was performed in adult transplant recipients and further studies in the pediatric population are needed.

The most important implication of our study is that RZV is a viable option to generate a humoral and cell-mediated response and potentially prevent primary varicella in immunosuppressed persons who did not receive adequate pretransplant varicella vaccination and remain at risk for primary varicella. Further studies will need to determine the efficacy of RZV in preventing varicella infection which will likely require multicenter collaborations.

REFERENCES

1. Feldhoff CM, Balfour HH Jr, Simmons RL, et al. Varicella in children with renal transplants. *J Pediatr*. 1981;98:25–31.
2. Lynfield R, Herrin JT, Rubin RH. Varicella in pediatric renal transplant recipients. *Pediatrics*. 1992;90:216–220.
3. Fehr T, Bossart W, Wahl C, et al. Disseminated varicella infection in adult renal allograft recipients: four cases and a review of the literature. *Transplantation*. 2002;73:608–611.
4. Gourishankar S, McDermid JC, Jhangri GS, et al. Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. *Am J Transplant*. 2004;4:108–115.
5. Fuks L, Shitrit D, Fox BD, et al. Herpes zoster after lung transplantation: incidence, timing, and outcome. *Ann Thorac Surg*. 2009;87:423–426.
6. Rubin LG, Levin MJ, Ljungman P, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. *Clin Infect Dis*. 2014;58:309–318.
7. Danziger-Isakov L, Kumar D; AST ID Community of Practice. Vaccination of solid organ transplant candidates and recipients: Guidelines from the American society of transplantation infectious diseases community of practice. *Clin Transplant*. 2019;33:e13563.
8. Lal H, Cunningham AL, Godeaux O, et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. *N Engl J Med*. 2015;372:2087–2096.
9. Vink P, Ramon Torrell JM, Sanchez Fructuoso A, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: A phase 3, randomized clinical trial. *Clin Infect Dis*. 2020;70:181–190.
10. Stadtmauer EA, Sullivan KM, Marty FM, et al. A phase 1/2 study of an adjuvanted varicella-zoster virus subunit vaccine in autologous hematopoietic cell transplant recipients. *Blood*. 2014;124:2921–2929.

11. Berkowitz EM, Moyle G, Stellbrink HJ, et al. Safety and immunogenicity of an adjuvanted herpes zoster subunit candidate vaccine in HIV-infected adults: a phase 1/2a randomized, placebo-controlled study. *J Infect Dis.* 2015;211:1279–1287.
12. Chlibek R, Bayas JM, Collins H, et al. Safety and immunogenicity of an AS01-adjuvanted varicella-zoster virus subunit candidate vaccine against herpes zoster in adults ≥ 50 years of age. *J Infect Dis.* 2013;208:1953–1961.
13. Cunningham AL, Heineman TC, Lal H, et al. Immune responses to a recombinant glycoprotein E herpes zoster vaccine in adults aged 50 years or older. *J Infect Dis.* 2018;217:1750–1760.
14. Reynolds MA, Kruszon-Moran D, Jumaan A, et al. Varicella seroprevalence in the U.S.: data from the National Health and Nutrition Examination Survey, 1999-2004. *Public Health Rep.* 2010;125:860–869.
15. Moris P, van der Most R, Leroux-Roels I, et al. H5N1 influenza vaccine formulated with AS03 A induces strong cross-reactive and polyfunctional CD4 T-cell responses. *J Clin Immunol.* 2011;31:443–454.
16. Salvadori M, Holzer H, de Mattos A, et al. Enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolate mofetil in de novo renal transplant patients. *Am J Transplant.* 2004;4:231–236.
17. Suresh S, Upton J, Green M, et al. Live vaccines after pediatric solid organ transplant: Proceedings of a consensus meeting, 2018. *Pediatr Transplant.* 2019;23:e13571.
18. Litwin V, Jackson W, Grose C. Receptor properties of two varicella-zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. *J Virol.* 1992;66:3643–3651.
19. Zhu Z, Gershon MD, Hao Y, et al. Envelopment of varicella-zoster virus: targeting of viral glycoproteins to the trans-Golgi network. *J Virol.* 1995;69:7951–7959.

20. Geel AL, Landman TS, Kal JA, et al. Varicella zoster virus serostatus before and after kidney transplantation, and vaccination of adult kidney transplant candidates. *Transplant Proc.* 2006;38:3418–3419.
21. Chaves Tdo S, Lopes MH, de Souza VA, et al. Seroprevalence of antibodies against varicella-zoster virus and response to the varicella vaccine in pediatric renal transplant patients. *Pediatr Transplant.* 2005;9:192–196.
22. Breuer J, Schmid DS, Gershon AA. Use and limitations of varicella-zoster virus-specific serological testing to evaluate breakthrough disease in vaccinees and to screen for susceptibility to varicella. *J Infect Dis.* 2008;197 Suppl 2:S147–S151.
23. Haberthur K, Engelmann F, Park B, et al. CD4 T cell immunity is critical for the control of simian varicella virus infection in a nonhuman primate model of VZV infection. *PLoS Pathog.* 2011;7:e1002367.
24. Kleemann P, Distler E, Wagner EM, et al. Varicella-zoster virus glycoproteins B and E are major targets of CD4+ and CD8+ T cells reconstituting during zoster after allogeneic transplantation. *Haematologica.* 2012;97:874–882.
25. Polcicova K, Goldsmith K, Rainish BL, et al. The extracellular domain of herpes simplex virus gE is indispensable for efficient cell-to-cell spread: evidence for gE/gI receptors. *J Virol.* 2005;79:11990–12001.
26. Godfrey DI, Rossjohn J, McCluskey J. The fidelity, occasional promiscuity, and versatility of T cell receptor recognition. *Immunity.* 2008;28:304–314.
27. Natori Y, Shiotsuka M, Slomovic J, et al. A double-blind, randomized trial of high-dose vs standard-dose influenza vaccine in adult solid-organ transplant recipients. *Clin Infect Dis.* 2018;66:1698–1704.
28. Shodell M, Shah K, Siegal FP. Circulating human plasmacytoid dendritic cells are highly sensitive to corticosteroid administration. *Lupus.* 2003;12:222–230.

29. Katerinis I, Hadaya K, Duquesnoy R, et al. De novo anti-HLA antibody after pandemic H1N1 and seasonal influenza immunization in kidney transplant recipients. *Am J Transplant*. 2011;11:1727–1733.
30. Cohet C, Haguinet F, Dos Santos G, et al. Effect of the adjuvanted (AS03) A/H1N1 2009 pandemic influenza vaccine on the risk of rejection in solid organ transplant recipients in England: a self-controlled case series. *BMJ Open*. 2016;6:e009264.
31. Dos Santos G, Haguinet F, Cohet C, et al. Risk of solid organ transplant rejection following vaccination with seasonal trivalent inactivated influenza vaccines in England: A self-controlled case-series. *Vaccine*. 2016;34:3598–3606.

FIGURE LEGENDS

Figure 1. Study design in terms of vaccine doses and study bloodwork.

RZV: recombinant zoster vaccine

Figure 2. Study flowchart.

SOT: Solid Organ Transplantation; VZV: Varicella-zoster virus; IgG: Immunoglobulin G;

HIV: Human Immunodeficiency virus; NYD: Not yet determined.

Figure 3. Evolution of anti-gE (3A), anti-gE avidity (3B) and gpELISA antibody (3C) after vaccine doses.

ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G

Figure 4. Frequency of antigen-specific cytokine-producing CD4⁺ (4A) and CD8⁺ (4B) T-cells after stimulation with a VZV peptide pool.

Results are expressed as median number of antigen-specific cytokine-producing CD4⁺ cells/10⁶ CD4⁺ T-cells (4A) and CD8⁺ cells/10⁶ CD8⁺ T-cells (4B).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table 1. Characteristics of Study Population

<u>Demographics</u>		
Patients, n		23
Male, n (%)		11 (47.8)
Median age at enrollment, yrs (IQR)		38.0 (31.3-55.8)
Median time since transplant, yrs (IQR)		3.8 (1.4-6.4)
<u>Race</u>		
White		20 (87.0)
Black		2 (8.7)
Asian		1 (4.3)
<u>Organ, n (%)</u>		
Liver		8 (34.8)
Lung^a		7 (30.4)
Kidney		4 (17.4)
Combined^b		4 (17.4)
<u>Comorbidities</u>		
Median BMI, kg/m² (IQR)		23.9 (20.3-29.0)
Obesity (BMI>30), n (%)		4 (17.4)
Diabetes, n (%)		5 (21.7)
Kidney insufficiency (GFR<60 mL/min/1.73 m²), n (%)		9 (39.1)
<u>Immunosuppression</u>		

Mycophenolate, n (%)		18 (78.3)
	Median dose, mg/d (IQR) ^c	720 (720-1080)
Cyclosporine, n (%)		4 (17.4)
	Median trough level, µg/l (IQR)	269 (193-319)
Tacrolimus, n (%)		19 (82.6)
	Median trough level, µg/l (IQR)	7.7 (5.0-8.7)
Prednisone, n (%)		18 (78.3)
	Median dose, mg/d (IQR)	5 (5-10)
Azathioprine, n (%)		2 (8.7)
<u>Received in the last 6 months</u>		
ATG		0 (0)
Methylprednisolone		3 (13.0)
Basiliximab		1 (4.3)

IQR: interquartile range; BMI: body mass index; ATG: antithymocyte globulin; GFR: glomerular filtration rate

^aTwo lung transplant patients also had a kidney transplant

^bliver/kidney (n=2); kidney/pancreas (n=1); kidney/heart (n=1)

^cequivalent mycophenolate daily dose

Table 2. Frequency of reactions reported during the 7-day post-vaccination period

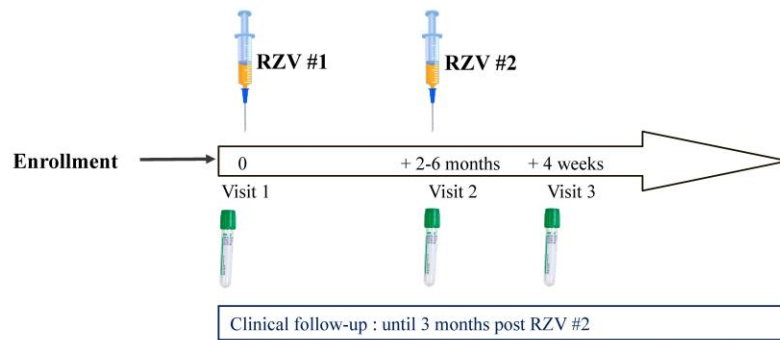
<i>Doses</i>		Frequency, n (%)	Median duration, days
<i>(N=38)</i>			<i>(IQR)</i>
<i>Injection-site reaction</i>			
	Redness	8 (21.1)	2.5 (1.3-3.8)
	>6cm	1 (2.6)	7 (-)
	Swelling	10 (26.3)	2 (2.0-3.3)
	>6cm	1 (2.6)	1 (-)
	Pain	29 (76.3)	3 (2-4)
	Severe	4 (10.5)	1.5 (1-2.8)
<i>Systemic reaction</i>			
	Fever >38°C	1 (2.6)	2 (-)
	Nausea and/or vomiting	3 (7.9)	2 (1-3)
	Severe	0	
	Fatigue	8 (21.1)	1.5 (1-3)
	Severe	0	

Myalgia	16 (42.1)	3 (1.0-4.0)
Severe	1 (2.6)	1
Headache	5 (13.2)	2 (1.0-4.5)
Severe	0	

IQR: interquartile range

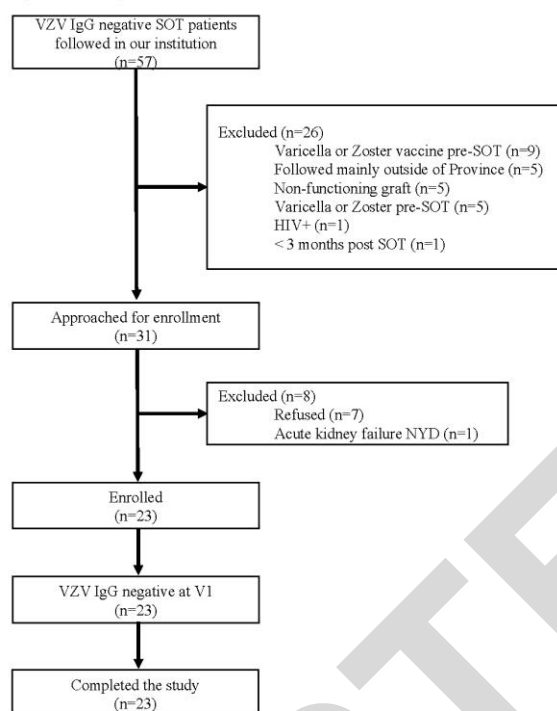
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Figure 1. Study design with vaccine doses and study bloodwork.



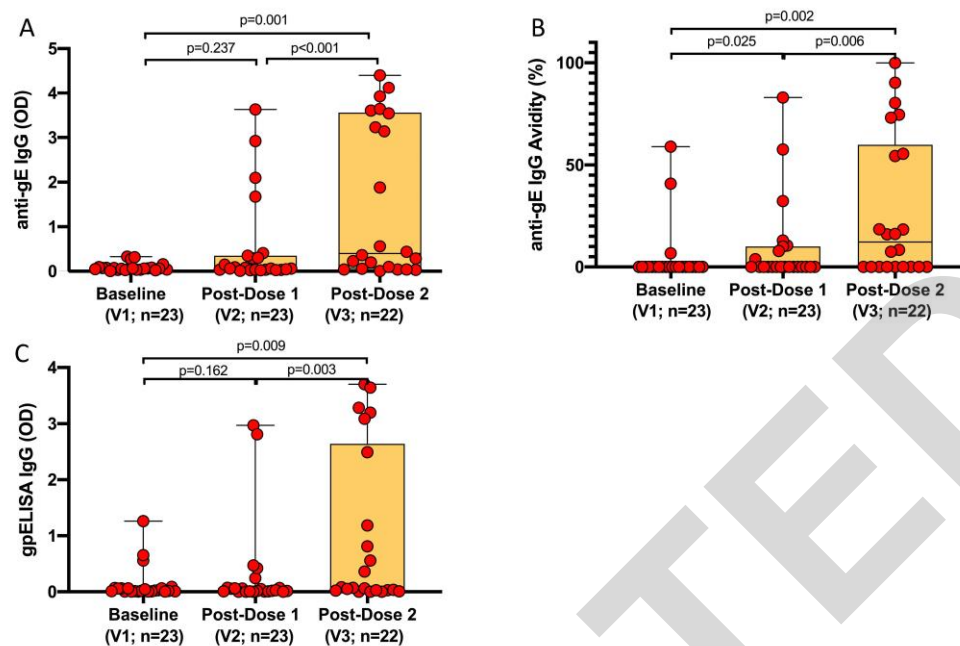
RZV: recombinant zoster vaccine

Figure 2. Study Flowchart



SOT: Solid Organ Transplantation; VZV: Varicella-zoster virus; IgG: Immunoglobulin G; HIV: Human Immunodeficiency virus; NYD: Not yet determined

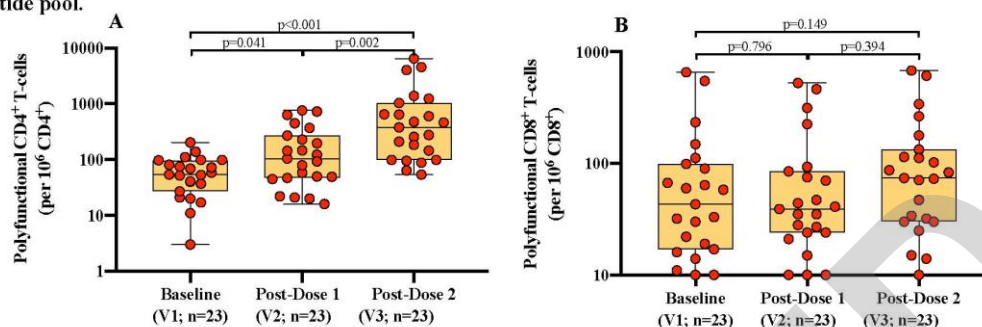
Figure 3. Evolution of anti-gE (3A), anti-gE avidity (3B) and gpELISA antibody (C) after vaccine doses.



The top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers represent the highest and lowest values.

ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; OD: optical density

Figure 4. Frequency of antigen-specific cytokine-producing CD4⁺ (4A) and CD8⁺ (4B) T-cells after stimulation with a VZV peptide pool.



Results are expressed as median number of antigen-specific cytokine-producing CD4⁺ cells/10⁶ CD4⁺ T-cells (4A) and CD8⁺ cells/10⁶ CD8⁺ T-cells (4B).

The top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers represent the highest and lowest values.